AD-A105 631 AMERICAN FOUNDATION FOR BIOLOGICAL RESEARCH ROCKVILLE MD F/G 6/5
CRYOPRESERVATION OF SCHISTOSOME LARVAE.(U)
SEP 81 M A STIREWALT, C S RICHARDS N00014-78-C-0081

UNCLASSIFIED NL

1 of 1
AD A
105631

END
DATE
FILMED
11-81
DTIC

LEVEL III

(12)

AD A105631

OFFICE OF NAVAL RESEARCH

(15) Contract No. N00014-78-C-0081

Task No. NR 204-072

(9) ANNUAL RE[redacted]T NO. 4, 30 Sep 80-1 Oct 81,

(6) Cryopreservation of Schistosome Larvae.

by

(12) 140

(10) M. A. Stirewalt

~~and~~

C. S. Richards

(11) Sep~~tember 19~~81

Reproduction in whole or in part is permitted for any purpose of the United States Government.

This document has been approved for public release; its distribution is unlimited.

NTIS GRA&I	X
DTIC TAB	
Unannounced	
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A	

412029

BACKGROUND

Significant protective immunity to *Schistosoma mansoni* develops in several strains (a) during a patent chronic infection, and (b) following immunization with highly irradiated viable cercariae. Since injection of highly irradiated cercariae does not lead to a patent schistosomal infection this type of immunization shows potential for human vaccine use.

Antigenic polymorphism within a single isolate of *Schistosoma mansoni* appears to affect the resistance of mice to second infection with various clones developed from the same isolate (Smith and Clegg, Parasitology 78: 311-321, 1979). Such antigenic polymorphism has important implications for experimental studies of immunity to schistosomiasis and also for the development of a vaccine.

Strains of *S. mansoni*, including isolates from different geographic areas, multiple isolates from some areas, and multiple substrains selected from individual isolates have been differentiated on the basis of infectivity for intermediate host snails. Maintained in this laboratory, this series of genetically different *S. mansoni* strains provides parasites for studies on variations in immunologic and pathologic aspects of different *S. mansoni* strains, and differences in cryopreservation qualities as means of storing them for vaccine production.

METHODOLOGY AND RESULTS

B. glabrata snails have been exposed, both as juveniles and adults, to a variety of strains of *S. mansoni*. By selection on the basis of susceptibility and isolation of snails as juveniles with reproduction by self-fertilization only, a series of inbred snail lines has been established showing different patterns of susceptibility. Exposures of snails from these characterized lines to miracidia of *S. mansoni* serves to differentiate genetic infectivity patterns in different parasite strains.

(1) Intraspecific genetic variations in susceptibility for *S. mansoni* strains were compared in 20 stocks of *B. glabrata*, including 7 stocks from Puerto Rico. Most of these snail stocks show a juvenile-susceptible/adult-nonsusceptible relation to some strains of *S. mansoni*. Adult susceptibility to *S. mansoni* strain PR-1 is determined at a single locus, following simple Mendelian inheritance, with adult nonsusceptibility dominant. Crosses have demonstrated that adult susceptibility to *S. mansoni* strain PR-2 is determined by either a different gene or a third allele, again with nonsusceptibility dominant. Crosses in progress suggest that adult susceptibility to *S. mansoni* strain PR-TM-9 is determined by a different genetic factor with susceptibility dominant. The seven stocks of Puerto Rican *B. glabrata* show different patterns of susceptibility, including one stock which is nonsuceptible at any age,

or juvenile susceptible/adult nonsusceptible to some *S. mansoni* strains isolated from chronic cases from the same locality.

(2) Many of our snail stocks have been made available to Margaret Mulvey (Rutgers) who is performing electrophoretic studies on their allozyme patterns. Many of the snail stocks have also been sent to Dr. Timothy Yoshino (Univ. Oklahoma) for comparative studies on the chemical nature of the hemocyte surface in snails differing in *S. mansoni* susceptibility.

(3) Studies on intraspecific genetic variations in *S. mansoni* have been continued. Twenty-one strains are being maintained and compared, including: 13 of Puerto Rican origin, 2 from St. Lucia, one Egyptian, one from Kenya, three from Zaire, and one from Central African Republic. Selection for different substrains from the Zaire isolates is in progress.

(4) Three reciprocal worm crosses have been carried out and another is in progress. Two crosses involved *S. mansoni* PR-2 with PR-T13 and PR-TA-13. Exposures of several snail stocks to F_1, F_2, and backcross miracidia indicated that infectivity for some snail stocks is dominant and for others recessive. Apparently several genetic infectivity factors are involved in the snail parasite combinations tested. Crosses between PR-C-3 and PR-T-3 were of particular interest. These substrains were derived from a single chronic case isolate. Experiments by Dr. Cheever (LPD, NIAID, NIH) have demonstrated differences in immunogenicity in mice between PR-C-3 (low) and PR-T-3 (high). Results suggest that snail infectivity and mouse immunogenicity factors may be linked. F_1 and backcross worms will be tested for immunogenecity and selection will be carried out based on snail infectivity to determine if the immunogenicity difference is genetic and if the factors are indeed linked.

(5) Cercariae of several different strains of *S. mansoni* have been provided to Dr. Cheever for immunogenicity studies. Most strains have shown a high degree of cross reaction, but PR-C-3 and PR-T-3 were significantly different in immunogenetically (see above). Substrains selected from chronic case PR-9 showed good immunogenicity in unisexual infections.

(6) Cercariae and adults of genetically different *S. mansoni* strains have also been provided LPD, NIAID, NIH (Simpson) for DNA studies. Initial tests demonstrated significant differences in the DNA of some of the Puerto Rican *S. mansoni* strains.

DISCUSSION

Ten isolates of *S. mansoni* from three geographic areas have so far yielded 21 parasite strains by selection on the basis of infectivity for *B. glabrata* snails. These appear to include abcut 17 different snail infectivity patterns, suggesting several genetic factors occurring in various combinations. Electrophorectic studies (Fletcher and LoVerde) on 13 of the strains demonstrated allozyme differences. The results indicated the need for further genetic characterization of parasite strains used in biomedical research, and comparative studies on the influence of strain differences on immunology, pathology, epidemiology, etc. Strains from additional geographic areas should be tested, characterized, and compared.

Collaborative studies at Purdue University (Fletcher and LoVerde) suggested that isozyme variations for at least one enzyme system, lactatedehydrogenase, in our *S. mansoni* strains were associated with variations in snail infectivity. Such studies should be continued. Collaborative electrophoretic studie. at Rutgers (Mulvey) are being carried out on the various snail stocks.

Most populations of *B. glabrata* studied to date demonstrate a juvenile-susceptible/adult-nonsusceptible relation to at least some *S. mansoni* strains. In most field situations juvenile snails are numerous enough to support transmission if susceptible. Since many field collections include only adults, this could lead to a misconception as to the transmission potential of the populations. Further studies on the relation of snail age to susceptibility should be carried out.

Strain differences in immunogenicity and development of good immunogenicity in unisexual infections (Cheever) need further study, including testing descendents of worm crosses to the clarify the genetics involved.

SIGNIFICANT ACCOMPLISHMENTS

1) A wide range of intraspecific genetic variations has been demonstrated in both *S. mansoni* and *B. glabrata*: 21 strains of *S. mansoni* showing about 17 different patterns of snail infectivity, and 20 stocks of *B. glabrata* showing about 16 different patterns of parasite susceptibility are under study.
2) Snail crosses have demonstrated several genetic factors (or alleles) determining adult susceptibility. In two of these nonsusceptibility is dominant, while in a third susceptibility appears to be dominant.
3) Seven Puerto Rican stocks of *B. glabrata* are being compared, and all seem to differ in susceptibility patterns; ranging from one stock susceptible to most *S. mansoni* strains at any age, to one that is nonsusceptible to some Puerto Rican *S. mansoni* strains at any age, juvenile susceptible/adult nonsusceptible to some, and susceptible to some. Variable adult susceptibility to some Puerto Rican *S. mansoni* strains occurs in some of the Puerto Rican *B. glabrata* stocks.

4) *S. mansoni* crosses have demonstrated several different snail infectivity factors, in some of which infectivity is dominant, in others lack of infectivity.
5) Mouse immunogenicity studies by Cheever (LPD, NIAID, NIH) have demonstrated a significant difference between two *S. mansoni* substrains from the same chronic case isolate (PR-C-3, low: PR-T-3, high). Substrains of another chronic case isolate, PR-9, showed good immunogenicity in unisexual infections.
6) Studies by Simpson (LPD, NIAID, NIH) showed differences in the DNA of Puerto Rican *S. mansoni* strains differing in snail infectivity.

PUBLICATIONS

1. Fletcher, M. LoVerde, P. T., and Richards, C. S.: Electrophoretic characterization of strains of *Schistosoma mansoni* from Puerto Rico and St. Lucia selected for high or low infectivity to the intermediate host. Exp. Parasit. (submitted, 1980).

2. LoVerde, P. T. Gherson, J., and Richards, C. S.: Reaction against self in an invertebrate? J. Invert. Pathol., (submitted, 1980).

3. LoVerde, P. T., Gherson, J., and Richards, C. S.: Amebocytic accumulations in *Biomphalaria glabrata*: Fine stucture. J. Invert. Pathol, (submitted, 1981).

4. Kasschau, M. R. and Richards, C. S.: Concentration changes in free amino acid pools during the life cycle of *Schistosoma mansoni*. (submitted, 1980).

5. Sullivan, J. T., Richards, C. S., Lie, K. J., and Heyneman, D.,: *Ribeiroia marini*: acquited resistance in *Biomphalaria glabrata* induced by irradiated miracidia. Exp. Parasit. (in press, 1980).

6. Sullivan, J. T., Richards, C. S. Lie, K. J., and Heyneman, D.,: Protection of *Schistosoma mansoni*, NIH-SM-PR-2 strain, in nonsusceptible *Biomphalaria glabrata* by *Echinostoma paraensei*. Internat. J. Parasit., (in press, 1980).

7. Sullivan, J. T., & Richards, C. S.: *Schistosoma mansoni*, NIH-SM-PR-2 strain in susceptible and nonsusceptible stocks of *Biomphalaria glabrata*: Comparative histology. Jour. Parasit. (in press, 1980).

8. Sullivan, J. T. & Richards, C. S.: Increased susceptibility to infection with *Ribeiroia marini* in *Biomphalaria glabrata* exposed to X-Rays. Internat. J. Parasit. (in press, 1980).

9. Sullivan, J. T. & Richards, C. S.: Comparative mortality of a laboratory and field stock of *Biomphalaria glabrata* exposed to four molluscicides. Bull. WHO, (submitted, 1980).

10. Sullivan, J. T. Richards, C. S., Lloyd, H. A., & Krishna, G.: Anacordic acid: molluscicide in cashe nut shell liquid. Planta. Medica. (in press, 1980).

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER 4	2. GOVT ACCESSION NO. AD-A105	3. RECIPIENT'S CATALOG NUMBER 632
4. TITLE (and Subtitle) CRYOPRESERVATION OF SCHISTOSOME LARVAE		5. TYPE OF REPORT & PERIOD COVERED Annual: 30 September 1980-1 October 1981
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) M. Stirewalt and C. S. Richards		8. CONTRACT OR GRANT NUMBER(s) ONR N00014-78-C-0081
9. PERFORMING ORGANIZATION NAME AND ADDRESS American Foundation for Biological Research 12111 Parklawn Drive Rockville, Maryland 20852		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS NR 204-072
11. CONTROLLING OFFICE NAME AND ADDRESS Procurement Contract Officer Office of Naval Research (443) Dept. of the Navy, Washington, D. C. 20360		12. REPORT DATE
		13. NUMBER OF PAGES 5
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report)
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Standard Distribution Lists

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Schistosoma mansoni; trematode; *Biomphalaria glabrata;* susceptibility of *B. glabrata;* genetics of *B. glabrata;* genetics of *S. mansoni;* immunogenicity.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A wide range of intraspecific genetic variations has been demonstrated in both *S. mansoni* and *B. glabrata:* 21 strains of *S. mansoni* showing 17 different patterns of snail infectivity, and 20 stocks of *B. glabrata* with 16 different patterns of parasite susceptibility are under study. Snail crosses demonstrated several genetic factors determining adult susceptibility. Seven Puerto Rican stocks of *B. glabrata* apparently differ genetically in susceptibility patterns. *S. mansoni* crosses have demonstrated several different snail infectivity factors, some dominant and some recessive. Mouse immunogenicity studies have demonstrated a significant difference between two *S. mansoni*

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

S/N 0102-LF-014-6601

continued

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

Abstract continued:

substrains from an isolate from a Puerto Rican chronic case, one substrain giving high protection, one low. *S. mansoni* substrains from a Puerto Rican chronic case showed good immunogenicity in unisexual infections.

A

OFFICE OF NAVAL RESEARCH
MICROBIOLOGY PROGRAM
STANDARD DISTRIBUTION LIST

Number of Copies:

(12)	Administrator, Defense Technical Information Center Cameron Station Alexandria, VA 22314
(6)	Director, Naval Research Laboratory Attn: Technical Information Division Code 2627 Washington, D.C. 20375
(3)	Office of Naval Research Department of the Navy Code 443 800 N. Quincy Street Arlington, VA 22217
(1)	Commanding Officer (Code 00) Naval Medical Research & Development Command National Naval Medical Center Bethesda, MD 20014
(1)	Naval Medical Research & Development Command Code 46 National Naval Medical Center Bethesda, MD 20014
(2)	Technical Reference Library Naval Medical Research Institute National Naval Medical Center Bethesda, MD 20014
(2)	Bureau of Medicine and Surgery Navy Department Code MED 314 Washington, D.C. 20372
(1)	Office of Naval Research Eastern/Central Regional Office Building 114, Section D 666 Summer Street Boston, MA 02210

Enclosure (3)

STANDARD DISTRIBUTION LIST (Cont'd)

Number of Copies:

(1)	Office of Naval Research Branch Office 536 South Clark Street Chicago, IL 60605
(1)	Office of Naval Research Western Regional Office 1030 East Green Street Pasadena, CA 91106
(1)	Commanding Officer U.S. Naval Medical Research Unit #2 APO, San Francisco 96528
(1)	Commanding Officer U.S. Naval Medical Research Unit #3 FPO, New York 09527
(1)	Officer in Charge Submarine Medical Research Laboratory U.S. Naval Submarine Base, New London Groton, CT 06342
(1)	Scientific Library Naval Biosciences Laboratory Naval Supply Center Oakland, CA 94625
(1)	Scientific Library Naval Aerospace Medical Research Institute Naval Aerospace Medical Center Pensacola, FL 32512
(1)	Commander, Naval Air Development Center Attn: Code 6003 Warminster, PA 18974
(1)	Commanding General U.S. Army Medical Research & Development Command Fort Detrick Frederick, MD 21701 Attn: MEDDH-Sr

STANDARD DISTRIBUTION LIST (Cont'd)

Number of Copies:

(1)	Director of Life Sciences Air Force Office of Scientific Research Bolling Air Force Base Washington, D.C. 20032
(1)	STIC-22 4301 Suitland Road Washington, D.C. 20390
(1)	Director Walter Reed Army Institute of Research Walter Reed Army Medical Center Washington, D.C. 20012

DATE
ILMED
-8